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Prognostic Value of High Mobility Group Protein A2 (HMGA2) over-expression in Cancer Progression

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Abstract

The high mobility group A2 (HMGA2; also called HMGI-C) gene is an architectural transcription factor that belonging to the high mobility group AT-hook (HMGA) gene family. HMGA2 is aberrantly regulated in several human tumors. Over-expression of HMGA2 is correlated with a higher risk of metastasis and an unfavorable prognosis in patients with cancer. We performed a meta-analysis to determine the clinic-pathological and prognostic value of HMGA2 overexpression in different human tumors. A comprehensive literature search was performed using PubMed, Embase, Cochrane Library, Scopus, MEDLINE, Google Scholar and ISI Web of Science. Hazard ratios (HRs)/odds ratios (ORs) and their 95% confidence intervals (CIs) were used to assess the strength of the association between HMGA2 expression and overall survival (OS)/progression free survival (PFS)/disease free survival (DFS). A total of 5319 patients with 19 different types of cancer from 35 articles were evaluated. Pooled data analysis indicated that increased HMGA2 expression in cancer patients predicted a poor OS (HR = 1.70; 95% CI = 1.6–1.81; *P*<0.001; fixed-effect model). In subgroup analyses, high HMGA2 expression was particularly associated with poor OS in individuals with gastrointestinal (GI) cancer (HR=1.89, 95% CI: 1.83-1.96; fixed-effect model) and HNSCC cancer (HR-1.78, 95%CI: 1.44-2.21; fixed-effect model). Over-expression of HMGA2 was associated with vascular invasion (OR = 0.16, 95% CI = 0.05-0.49; *P* = 0.001) and lymphatic invasion (OR =1.89, 95% CI = 1.06-3.38; *P*= 0.032). Further studies should be conducted to validate the prognostic value of HMGA2 for patients with GI cancers.

Keywords: High mobility group A2, Prognosis, Recurrence, Survival, Invasion

1.Introduction

The effective management of patients with cancer requires early diagnosis and intervention (Belge et al., 2008; Aghabozorgi et al., 2018). There have been considerable efforts to identify the underlying molecular mechanism and novel sensitive and specific tumor biomarkers linked with some cancers, for the purpose of screening, diagnosing, determining the prognosis, and monitoring of therapy (Bahrami et al., 2018; Binabaj et al., 2018; Khayami et al., 2018).

The high mobility group A2 (HMGA2; also named HMGI-C) gene is an architectural transcription factor which belongs to the high mobility group AT-hook (HMGA) gene family. HMGA2 encodes for a non-histone chromatin protein which has no natural transcriptional function, but has the ability to modulate gene expression, replication and repair by binding to the minor groove of AT-rich region of DNA and consequently altering the chromatin structure (Fedele et al., 2002; Sgarra et al., 2004). It additionally increases the recruitment of other transcriptional regulators and binds to numerous protein complexes located on promoter/enhancer sites, creating the so-called enhanceosome (Watanabe et al., 2009). In humans, the HMGA2 protein is encoded by a gene located on chromosome locus 12q14-15. It is a 109 amino acid protein that acts as chromatin remodeling factor. HMGA2 is highly expressed during embryogenesis, although its expression is silenced in most adult organs (Chiappetta et al., 1996). When the HMGA2 gene is knock-out in mice it causes a pygmy phenotype which is identified by mesenchymal tissue hypoplasia, which provides evidence for the crucial role of HMGA2 in mammalian growth, differentiation and development (Zhou et al., 1995). As an oncogene, a high level of HMGA2 expression has been found to be involved in tumorigenesis in adults; in differentiated cells, HMGA2 expression is usually absent (Langelotz et al., 2003; Sarhadi et al., 2006; Shell et al., 2007). HMGA2 reactivation is a hallmark of different epithelial or interstitial benign and malignant tumors. HMGA2 is an important molecular target for chromosomal aberrations. Moreover, HMGA2 participates in the process of

carcinogenesis via disruption of partner gene transcription, truncation, or production of fusion genes coding chimeric transcripts (Fusco and Fedele, 2007). Deregulation of the gene through rearrangements of chromosome 12q13-15 and also high-expression of the full-length HMGA2 protein leads to various benign or neoplastic mesenchymal tumors (Fedele et al., 2001). Furthermore, a high level of HMGA2 expression in human carcinomas has been found to be related with epithelial–mesenchymal transition (EMT), in which epithelial or epithelial-like cells acquire mesenchymal characteristics (Miyazawa et al., 2004).

The HMGA2 enhances self-renewing in stem cells by reducing p16 amplification and cellular senescence. Furthermore, HMGA protein over-expression is associated with neoplastic transformation (Wood et al., 2000).

The stimulatory impact of HMGA2 on cancer cell behavior such as proliferation, invasion and metastasis has been reported in many types of malignancies (Li et al., 2014; Zhou et al., 2014). However, mis-expression of HMGA2 expression has been detected in different human epithelial-type tumors, such as breast cancers(BC) (Rogalla et al., 1997), pancreatic cancer(Abe et al., 2003), lung cancers(LC)(Sarhadi et al., 2006), ovarian cancer (OC) (Shell et al., 2007), oral squamous cell carcinomas (OSCC) (Miyazawa et al., 2004), hepatocellular cancer (HCC)(Luo et al., 2013), gastric cancer (GC) (Motoyama et al., 2008), malignant gliomas (Gong et al., 2014), pituitary adenomas (Fedele et al., 2006; De Martino et al., 2009) and thyroid carcinomas(Belge et al., 2008). Expression of HMGA2 may be related to Dukes stage, tumor grade (Piscuoglio et al., 2012) and metastasis of tumor cells (Huang et al., 2009). A line of evidence reveals that highly HMGA2 expression is indicative of tumor progression, poor prognosis and response to therapies for any cancer type (Wang et al., 2011; Califano et al., 2014). But, the underlying mechanism by that HMGA2 is regulated and the practical potency of HMGA2 within metastasis are still not completely established.

Despite many years of research and hundreds of studies performed on cancer biomarkers in oncology, the number of indicators that have emerged as clinically valuable is

small and not promising. Because the most of available prognostic markers have efficacy in a limited number of cancer patients, a novel and common molecular biomarker is needed for prognosis of cancer to attain higher survival rates. Thus, we performed a meta-analysis to quantify the clinicopathological and prognostic value of HMGA2 overexpression in different human tumors.

2. Material and Methods

2.1. Study Strategy

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009). The online databases PubMed, Embase, Cochrane Library, MEDLINE, Scopus, Google Scholar and ISI Web of Science were searched using medical subject headings (MeSH) terms and free-text words to enhance the search sensitivity. The following search terms were applied: “HMGA2, High-mobility group A2” and “cancer, tumor, malignancy, neoplasm” and “prognosis, prognostic, survival, mortality”. The research topic and publication language were restricted to human and English respectively. The reference lists of the selected studies and related review articles were re-screened to recognize further relevant papers. The articles entered in this meta-analysis were published up to September 2018.

2.2. Inclusion and exclusion criteria

The inclusion criteria for the present study are mentioned as follows: (1) valid tumor, node, metastasis (TNM) stage, tumor differentiation, metastasis, lymph/vascular invasion, recurrence data, as well as sufficient survival data, such as hazard ratio (HR) or relative ratio (RR) with 95% confidence intervals (CI), overall survival (OS), disease free survival (DFS), relapse-free survival (RFS) and progression free survival (PFS) was estimated using the univariate/multivariate analyses or Kaplan-Meier method; (2) and analyzed the association between HMGA2 expression and prognosis or pathological parameters; (3) prognosis of cancer patients was reported by OS; (4) HMGA2 expression was sub-divided into high (+) and low (-) expression groups; (5) similar themes and methods, and (6) any other beneficial and relevant details could be obtained from the full-text study. Exclusion criteria consist of (1) duplicate researches, letters, conference papers, case reports and reviews; (2) in vitro studies and animal experiments;

2.3. Data extraction

Full text of each study was inspected in detail. Two independent investigators (MM and AB) reviewed all included publications and obtained the following data from each: name of first author, publication year, country, cancer type, type of specimen, sample size, patients information, tumor grade, detection method, clinicopathological features and HRs of HMGA2 for OS/PFS/RFS/DFS with 95% confidence intervals (CIs). Any presence of discrepancy between researchers was resolved by debate until reaching consensus.

2.4. Statistical analysis

The desirable value used in current meta-analysis to estimate the causal relationship between HMGA2 expression and cancer prognosis was the HR. Whilst, pooled odds ratio (OR) with 95% CI were suitable for the association between HMGA2 expression and clinical features. When the HR>1, a worse prognosis for patients was indicated by positive HMGA2 expression. If the study provided the HR/RR with 95% CI, we used it for analysis. In case a study did not supply the HR/RR with 95% CI, these indices were quantified from Kaplan–Meier curves by valid method(Jafari et al., 2018). If HRs were provided for both multivariate and univariate analyses, the multivariate were adopted. A test of heterogeneity of combined HRs was performed using Higgins I-squared statistic(Higgins and Thompson, 2002). If I^2 <50%, a fixed-effect model was used for the calculation of the pooled HRs; unless, a random-effect model was more appropriate(Mantel and Haenszel, 1959). When I^2>50%, subgroup analysis or the sensitivity method which exclude one study constructed by Patsopoulos and collagenous (Patsopoulos et al., 2008) was used to answer the query of heterogeneity. We used OR with 95% CI to assess the association between HMGA2 expression and clinical parameters including the stage, differentiation, invasion, recurrence, metastasis as well as the patient's sex. A funnel plot (qualitative) and Egger's linear regression tests (quantitative) were performed to determine whether there was publication bias. All P values were two sided test and a P value < 0.05 was

set as statistically significant. Comprehensive a meta-analysis software version 2 (Biostat, Inc., Englewood, NJ) was used to conduct the meta-analysis.

3. Results

3.1. Search results

The detailed search strategy is summarized in Figure 1. A total of 749 papers from the online database were found that related the association between HMGA2 expression and different human tumors. Following duplicate deletion, 629 studies remained for titles and abstracts screening; subsequently, 519 studies were removed in this phase because of not meet the inclusion criteria. After that, 110 potentially relevant research articles were selected for full-text evaluation, and 77 articles were excluded due to the No human studies, review article and assessment other biomarkers. Finally, 35 studies were included in the present meta-analysis. The search strategy and selection process of studies is shown in Figure 1.

3.2. Characteristics of eligible studies

The eligible studies were published between 2004 and 2017. The types of specimens gathered from patients consist of formalin-fixed and paraffin-embedded (FFPE; n =25); frozen tissue (n=2), tissue (n=3), whole blood (n=1) and tissue microarray (TMA; n=4). Most of studies (n=32) used the immunohistochemistry (IHC) method to quantify the HMGA2 expression level, but two studies used quantitative real-time-PCR (qRT-PCR) and another applied western blot. Concerning country of study, 18 studies were performed in China, 4 studies in Taiwan,3 studies in Japan and Korea, 2 studies performed in Italy and one study carried out in each of these countries: Denmark, Norway, Finland, Germany and USA. The sample size in eligible studies ranged from 23 to 330. Among these 33 studies, 18 different tumor types were included in this meta-analysis, including 2 bladder cancer (BLD)(Yang et al., 2011; Ding et al., 2014),1 BC(Wu et al., 2016), 1 clear cell renal cell carcinoma (CCRCC)(Na et al., 2016), 1 cholangiocarcinoma(Lee et al., 2014), 6 colorectal cancer (CRC)(Wang et al., 2011; Rizzi et al., 2013; Liu et al., 2015; Liu et al., 2016; Yu et al., 2016), 1 esophageal squamous cell carcinoma(ESCC)(Wei et al., 2016), 1 glioblastoma (GBM)(Zhang et al., 2018), 5 GC(Motoyama

et al., 2008; Kong et al., 2014; Jun et al., 2015; Lee et al., 2015; Dong et al., 2017); 1 gallbladder cancer(Zou et al., 2012), 1 HCC(Wu et al., 2012), 2 head and neck squamous cell carcinoma (HNSCC)(Yamazaki et al., 2013; Günther et al., 2017), 2 LC(Sarhadi et al., 2006; Gao et al., 2017), 1 melanoma(Raskin et al., 2013), 2 NSCLC(Eide et al., 2016; Guo et al., 2018), 1 nasopharyngeal carcinoma (NPC)(Xia et al., 2015), 4 oral squamous cell carcinoma (OSCC)(Miyazawa et al., 2004; Chang et al., 2015; Fang et al., 2017; Ren et al., 2017) , 2 OV(Califano et al., 2014; Kim et al., 2015), 1 pancreatic ductal adenocarcinoma (PDAC)(Strell et al., 2017), and 1 tongue squamous cell carcinoma(TSCC)(Zhao et al., 2016). Main characteristics of included studies are shown in Table 1.

3.3. Correlation of HMGA2 expression with clinicopathological parameters

We evaluated the relationship between HMGA2 expression and clinicopathological characteristics in all of the cancer patients. Because the I^2 value for all features was more than 50.0%, the random-effects model was applied for data pooling. The final results from the meta-analysis showed that HMGA2 over expression was significantly associated with vascular invasion (OR = 0.16, 95% CI = 0.05-0.49, *P* =0.001), lymphatic invasion (OR =2.14, 95% CI = 1.184-3.864, *P*= 0.012) and gender (OR=3.84, 95% CI =1.99-7.43, *P*<0.001). However, higher HMGA2 expression was not related with TNM stage (OR=1.99, 95% CI = 0.48-8.29, *P* =0.344), tumor differentiation (OR =1.59, 95% CI=0.801-3.144; *P* = 0.186), distant metastasis (OR=0.32, 95% CI = 0.05-1.95; *P*= 0.218), T stage (OR = 1.83, 95% CI = 0.71-4.71; *P* =0.212), recurrence (OR=2.10, 95% CI = 0.34-12.99; *P* =0.424) and neural invasion (OR=0.47, 95% CI =0.15-1.47; *P* = 0.194).

3.3. Correlation of HMGA2 over expression and survival

Twenty-five studies were included in the OS analysis, and the fixed-effects model was conducted (after excluding Gao et al. (Gao et al., 2017) and Dong et al. (Dong et al., 2017) due

high heterogeneity) due to low heterogeneity (*I2*=0.00%, *P*=32.37). Pooled data analysis indicated that increased HMGA2 expression in cancer patients predicted a shorter OS (HR=1.70, 95% CI = 1.60–1.81; *P*<0.001). In addition, we performed stratified analyses by categorizing studies into subgroups. In stratified analyses with tumor types, 12 studies reporting gastrointestinal(GI) cancers indicated that were particularly associated with worsen OS (HR=1.89, 95% CI:1.83-1.96; Figure 2 A). Other 8 studies revealed that HMGA2 exerted a significant effect on OS in HNC patients (HR=1.78, 95% CI: 1.44-2.21; Figure 2B), and 9 studies with other types of cancer yielded a similar result (HR=2.15, 95% CI: 1.65-2.80; Figure 2C). Furthermore, up-regulated HMGA2 was associated with unfavorable OS in both Asian (HR=1.77, 95% CI: 1.59-1.83) and Non-Asian population (HR=1.67, 95% CI: 1.40-1.98). Results from other stratified analyses were presented in Table 3.

As shown in Figure 3, 4 studies evaluated PFS. Due to the obvious heterogeneity between these studies (I^2 = 68.093%; *P* =0.014), the random effect model was used. The pooled data showed that a high level of HMGA2 was not significantly correlated with poorer PFS in cancer patients (HR = 1.48, 95% CI = 0.92-2.39, *P* = 0.105; Random effect-model; Figure 3A). For DFS analysis due to significant heterogeneity between these studies (I^2 = 63.608%; *P* =0.003), the random effect model was used. In addition, results showed that high expression of HMGA2 was correlated with shorter DFS (HR=2.134, 95%CI; 1.53-2.975 p=0.003: (Figure 3B). The result of Egger's test (p = 0.679), and following inspection of the symmetry of the funnel plot revealed evidence of publication bias across the studies (Figure 4).

3.4. Results of Sensitivity Analysis

The removal of two studies (Dong 2017 et al. (Dong et al., 2017) and Gao et al. (Gao et al., 2017)) had significant influence on the pooled HRs of the association between the HMGA2 expression and OS of cancer patients in pooled OS and subgroup of multivariate, studies conducted after year 2009 in the Asian region. Therefore these two studies were excluded from

in mentioned analysis because of their impact on the summary results. The I^2 in different subgroups decreased significantly, after removing these studies (Data not shown).

Discussion

HMGA2 is a non-histone DNA-binding protein and regulator of cell proliferation and differentiation which is one of the HMGA protein family members. It is an normal oncofetal protein highly regulated in embryonic tissues, while it is low/no detectable in adult differentiated tissues (Chiappetta et al., 1995). Recently, finding by multiple researches indicated that HMGA2 expression was correlated with the progression, aggression, dissemination and prognosis of some malignant tumors, and cancers with over-expression of HMGA2 being rather malignant, and prone to worse prognosis (Miyazawa et al., 2004; Chiappetta et al., 2008).

HMGA proteins have been observed to promote the function of the activator protein 1 (AP-1) complex which activates various target genes contributing in control of cell growth, proliferation, tumor formation and propagation(Angel and Karin, 1991). HMGA2 enhances human telomerase reverse transcriptase (hTERT) transcription to upgrade carcinogenesis, which is necessary for cancer cell development and self-renewal (Li et al., 2011). Notably, HMGA2 mRNA may be a molecular target for microRNAs, for example the let-7 family (Lee and Dutta, 2007; Mayr et al., 2007). Besides, HMGA2 binds with retinoblastoma (pRb) and promotes the E2F1 transcription factor function by displacing histone deacetylase 1 (Fedele et al., 2006).

Furthermore, HMGA2 potentially plays a role in the EMT process in which tumor cells lose their epithelial cells phenotype and switch to a more fibroblast-like morphology and retrieves their abilities of invasion, migration and/or proliferation in an uncontrolled manner, leading to tumor invasion and metastasis (Gao et al., 2012; Morishita et al., 2013). In the EMT process, cancer cells migrate from the originally site and invade the surrounding tissue, and then enters the circulation to constitute new proliferating colonies. A major hallmark of EMT, down-expression of the epithelial protein E-cadherin and over-expression of mesenchymal proteins such as vimentin, has been connected with metastatic propagation and patient survival in several solid tumor types (Luo et al., 2012; Smith et al., 2013; Tian et al., 2013). It has been recently reported that the nuclear factor-κB (NF-κB) signal pathway, Wnt/β-catenin pathway and

transforming growth factor β (TGFβ) mediated EMT process acts via stimulation of HMGA2 through the Smad cascade (Yang et al., 2016). Overall, HMGA2 has complicated activities, for instance, HMGA2 silencing was reported to elevate caspase-9 and P27 protein expression, and reduce Bcl-2 protein expression to motivate apoptosis and prevent proliferation through the phosphatidylinositol-3 kinases (PI3K)/Akt signaling cascade in GC (Wei et al., 2013). HMGA2 was found to be co-regulated with p53 in papillary serous carcinoma at an advanced stage (Wei et al., 2010). Similarly, aberrant expression of HMGA2 remarkably increased the phospho-p53 expression in BC cells after doxorubicin treatment which indicated the possible role of HMGA2 in the p53 pathway(Wu et al., 2016).

HMGA2 also controls the regulation of transcription factors families that play a role in EMT including Snail, Zinc finger E-box–binding (ZEB), Slug, and Twist (Thuault et al., 2006). Furthermore, extracellular matrix degradation is another element involved in the progression to invasion and metastasis(Wu et al., 2015). HMGA2 may suppress the expression of matrix metalloproteinase (MMP-2 and -9), and elevate the invasion capability (Yan et al., 2016). Interestingly, oncogenic RAS signaling axis considerably triggers EMT process in pancreatic cancer cells by promotion of HMGA2 expression(Watanabe et al., 2009). It has been shown that HMGA2 and EMT-markers are differentially regulated between nasopharyngeal carcinoma and adjacent non-tumor tissues(Xia et al., 2015).

In gastric cancer, ectopic HMGA2 expression induces protein alterations consistent with EMT and increased epithelial cell invasion and metastasis in laboratory and experimental models(Zha et al., 2013). In a similar way, in ovarian cancer cell lines, HMGA2 silencing partly inhibited the aggressive behavior of cancer cells, and led significant changes in the amplification of several EMT-related genes such as vimentin and E-cadherin(Wu et al., 2011). Furthermore, there is some evidence that the expression of HMGA2, E-cadherin, and vimentin is correlated with various aggressive behaviors, including N stage, T stage, TNM stage, 2-year metastasis and cancer recurrence(Ding et al., 2014; Xia et al., 2015). In the present meta-analysis,

expression of HMGA2 was associated with lymph invasion, gender and vascular invasion; however, no association was found with T stage, TNM stage and recurrence. These conflicting results between studies might be due to the different antibodies used and staining assessment methods, and the distinctive biological activities of HMGA2 in any type of tumors. Also, it is unclear, when HMGA2, is re-induced within malignant transformation.

In this analysis, 19 types of human malignancies encompassing 5319 patients were included. The meta-analysis results indicated that increased expression of HMGA2 was linked with poor OS and DFS in cancer patients. Moreover in the subgroup analysis for OS, we observed this relationship in GI, HNCC and other cancer subgroups. However, regarding the TNM stage, tumor differentiation, distant metastasis, T stage, recurrence and neural invasion we did not find any significant association. All these data support our opinion that the intensity of HMGA2 up-regulation is one of the main indicators of invasiveness of GI cancers, influencing the survival of GI cancer patients.

There are several limitations in the current meta-analysis. First, heterogeneity among studies was evident, which might be due to the discrepancies in tumor types, study regions and cut-off thresholds for positive and negative HMGA2 expression. Second, the number of eligible articles in some cancer types is relatively small, causing inadequacy of papers for subgroup analyses. However, more studies are required to reach a consensus for constant criteria for up-regulation of HMGA2. Negative or null associations may be publication bias. Subgroup analysis could not remove the heterogeneity between studies entirely. Fourth, certain studies did not report OS, PFS or DFS/RSS data directly, which were consequently obtained from Kaplan–Meier curves in some cases.

In conclusion, our meta-analysis showed that HMGA2 expression is an independent prognostic factor for OS and a novel potential therapeutic target in patients with GI cancers. HMGA2 expression is also associated with gender, lymph invasion and vascular invasion. The ectopic expression of HMGA2 may contribute in the tumorgenesis. Nevertheless, more

investigation is required to analyze and confirm the association between HMGA2 expression and prognosis for GI cancers patients.

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Figure and Table legends:

Figure 1. Flow diagram of search strategy.

Figure 2. Forest plot for the association between HMGA2 expression and OS. (**A**) all tumors; (**B**) gasterointestinal cancers; (**C**) head and neck cancer; (**D**) other type of cancer

Figure 3. Forest plot for the association between HMGA2 expression and (A) PFS; (B) DFS in all cancer patients

Figure 4. Funnel plot for publication bias test among studies.

Table 1. Main characteristics of included studies

Table 2. Meta-analysis of included studies according to clinicopathological features

Table 3. Subgroup analysis of pooled HR for overall survival

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